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14. ABSTRACT

Efforts in this project focused on H2/biofuels production and metabolism in eukaryotic algae and cyanobacteria. These research efforts led to over thirty-four publications in the three-year project performance period, including publications in the top tier journals Nature Communications and Science. A principal focus in this iteration of research was the development of new model systems for algal H2 and biofuels production. This led to the genome sequencing of Nannochloropsis gaditana and the development of genetic transformation protocols for this algal. These results were published in Nature Communications and this alga is poised to become a new biotechnologically relevant model system. We also successfully developed fatty acid and terpenoid biosynthesis/secretion modules in the cyanobacterium Synechococcus sp. PCC 7002. This very promising approach allows the transformation of sunlight to biofuels that are secreted and do not require costly phototroph harvesting, dewatering and deconstruction steps to obtain the biofuel precursors.

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RENEWABLE BIO-SOLAR HYDROGEN PRODUCTION: THE SECOND GENERATION (Part C)

Posewitz group (Colorado School of Mines)

Efforts in this project focused on $H_{2/}$biofuels production and metabolism in eukaryotic algae and cyanobacteria. Specific research thrusts included: (a) disrupting the hydrogenase enzymes in the green alga *Chlamydomonas reinhardtii*, (b) increasing the levels of algal cellular starch, (c) understanding the mechanism of [FeFe]-hydrogenase maturation, (d) exploring the diversity and evolution of algal H_2 metabolism, (e) elucidating algal fermentative metabolic networks, (f) exploring the biodiversity of H_2 production in phototrophs, (g) developing new model algal biofuel platforms and (g) carbon-based biofuels production and secretion in cyanobacteria. These research efforts led to over thirty-four publications in the three-year project performance period, including publications in the top tier journals *Nature Communications* and *Science*. A principal focus in this iteration of research was the development of new model systems for algal H_2 and biofuels production. This led to the genome sequencing of *Nannochloropsis gaditana* and the development of genetic transformation protocols for this algal. These results were published in *Nature Communications* and this alga is poised to become a new biotechnologically relevant model system. We also successfully developed fatty acid and terpenoid biosynthesis/secretion modules in the cyanobacterium *Synechococcus* sp. PCC 7002. This very promising approach allows the transformation of sunlight to biofuels that are secreted and do not require costly phototroph harvesting, dewatering and deconstruction steps to obtain the biofuel precursors. Lastly, we have improved and characterized the mechanisms of starch hyperaccumulation in *Chlamydomonas reinhardtii*, which can be used to make a variety of glucose based products.

The Nannochloropsis genus of algae is widely regarded as having among the most promising algal strains for salt-water biofuel production. As a part of this project we sequenced the genome and developed transformation protocols for a particularly promising strain, *Nannochloropsis gaditana* (CCMP526). Our data indicate that gene-knock ins and random mutants can be readily made and that this algal will be the focus of significant efforts to attain lipid-based biofuels. Another significant advance was the generation of starch hyperaccumulating mutants. By overexpressing the isoamlyase gene in Chlamydomonas, central metabolites are trafficked to starch biosynthesis at the expense of cell division and protein biosynthesis. This research was presented at the Society of Industrial Microbiology and a “top-poster” award. In Chlamydomonas, starch represents the major energy storage product. During anaerobic fermentation, glucose derived from starch provides the reductant for H_2 production. Moreover, reductant derived from starch oxidation has been shown to be a crucial factor in sustaining H_2 photoproduction. Starch oxidation can also drive anoxic, PSII independent H_2 photoproduction. Starch biosynthetic and catabolic pathways are therefore important targets for manipulation to improve hydrogenase activity. Importantly, this bioenergy carrier is synthesized in nutrient replete media, obviating the need to perform costly nutrient deprivation shifts to stimulate starch accumulation.

The third major focus of this project period was developing biofuel secretion for cyanobacteria. Using the euryhaline and rapid growing organism *Synechococcus* sp. PCC 7002, were able to achieve synthesis of fatty acids and terpenoid products that can be used as diesel fuels in this organism. C12 fatty acids, limonene (C10) and bisabolene (C15) was all synthesized and secreted by this cyanobacterium. Effort are now underway to improve titers and devise strategies to capture the secreted products.

In sum, the most significant advances were (a) development of *Nannochloropsis gaditana* into a model marine alga for biofuels production, (b) enabling starch hyperaccumulation under nutrient replete conditions, and (c) enabling biofuel secretion from cyanobacteria. Additional advances were made in discovering robust H_2 production in halophilic species of Tetraselmis, characterizing hydrogenase diversity in the Great Salt Lake, and elucidating Chlamydomonoas fermentative metabolic networks.

Publications

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